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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/371,648	08/10/99	YANAGIMACHI R	265036600070

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BARBARA E ARNDT
JONES DAY REAVIS & POGUE
NORTH POINT
901 LAKESIDE AVENUE
CLEVELAND OH 44114

EXAMINER	
PARAS JR, P	
ART UNIT	**PAPER NUMBER**
1632	9

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Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No.	Applicant(s)	
	09/371,648	YANAGIMACHI, RYUZO	
	Examiner	Art Unit	
	Peter Paras, Jr.	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-21 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-21 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

a)☐ All b)☐ Some * c)☐ None of the CERTIFIED copies of the priority documents have been:

1.☐ received.

2.☐ received in Application No. (Series Code / Serial Number) _____ .

3.☐ received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. & 119(e).

Attachment(s)

14) ☒ Notice of References Cited (PTO-892)
15) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
16) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
17) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
18) ☐ Notice of Informal Patent Application (PTO-152)
19) ☐ Other: .

DETAILED ACTION

Claim 16 is objected to because of the following informality: there are **two** periods at the end of the claim. Appropriate correction is required.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 9-13 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 9-13 contain the term "first time period" which does not convey any clear meaning. Is there a second period of time relevant to the method?

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-21 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of creating a transgenic embryo or a transgenic mouse which includes incubating a membrane-disrupted or a demembranated mouse sperm head with an exogenous DNA fragment to form a complex, microinjecting, with a piezo-electrically actuated microinjection apparatus, the

sperm-DNA complex into the cytoplasm of unfertilized mouse oocytes, culturing and implanting the surviving and dividing mouse embryos, analyzing the mouse embryos for the presence of the exogenous DNA fragment, does not reasonably provide enablement for a method of obtaining a transgenic embryo or creating transgenic animals, **of any and all species**, which includes "co-inserting", by any and all methods, a membrane-disrupted or demembranated sperm head of **any and all species** and an exogenous DNA fragment simultaneously either as a complex or separately into an unfertilized oocyte of **any and all species**, and analyzing the dividing and surviving embryos for the presence of the exogenous DNA fragment. Furthermore the specification is not enabled for "allowing the transgenic embryo **[of any and all species]** to develop into a live offspring" and "transplanting the transgenic embryo **[of any and all species]** into a surrogate mother **[of any and all species]**". The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Claims 1-20 read on any method of transferring a sperm head and exogenous DNA into an oocyte. At the time the claimed invention was made methods of transfer of a sperm head and exogenous DNA into oocytes to create transgenic embryos were unpredictable. Despite the specific embodiments taught by the specification; the method of the claimed invention not only encompasses transfer of a sperm head-exogenous DNA complex into oocytes by microinjection using a piezo-electrically actuated apparatus as a method of transfer; it encompasses all methods of "co-inserting" of a

sperm head and exogenous DNA into oocyte. As of the effective filing date of the claimed invention, the art of creating transgenic embryos by "co-inserting" a sperm and exogenous DNA into an oocyte was unpredictable with respect to the predictability of the incorporation of transgenes and the **result** of such incorporation in all species of animals. The specification fails to set forth parameters and conditions that would enable all methods of "co-inserting" sperm heads and exogenous DNA into an oocyte to create a transgenic embryo except by microinjection of a DNA-sperm head complex using a piezo-electrically actuated apparatus as a method of transfer. The specifcation provides no guidance or working examples that demonstrate methods of transferring sperm heads and exogenous DNA into an oocyte to create a transgenic embryo except by microinjection of a DNA-sperm head complex using a piezo-electrically actuated apparatus as a method of transfer. The term "co-inserting" can read on separate but simultaneous microinjections of DNA and sperm heads. Separate but simultaneous injections would require two injection needles in addition to a holding pipette. Under conditions where two injection needles are penetrating an oocyte, there is little chance of the oocyte surviving. Furthermore, there are no injection apparatus with the capacity to accommodate three micromanipulators, which would be necessary for separate but simultaneous "co-inserting" of DNA and sperm heads.

Thus in view of the breadth of the claims, lack of guidance provided by the specification, the absence of working examples, and the unpredictability of the art, one of skill in the art would have required an undue amount of experimentation to create any

transgenic embryo by any and all methods of "co-inserting" a sperm head and exogenous DNA into oocytes except by microinjection of a DNA-sperm head complex using a piezo-electrically actuated apparatus as a method of transfer, without a reasonable expectation of success.

Claims 1-15, 21 are drawn to the creation of transgenic embryos of any and all species using the method of the claimed invention. At the time the claimed invention was made generation of transgenic animals of any and all species using the method of the claimed invention was unpredictable. Despite the specific embodiments taught by the specification; the method of the claimed invention not only encompasses the creation of transgenic mouse embryos; it encompasses the creation of transgenic animal embryos of any and all species. As of the effective filing date of the claimed invention, the art of creating transgenic animal embryos of any and all species by transferring a sperm and exogenous DNA into an oocyte was unpredictable with respect to the predictability of the incorporation of transgenes and the **result** of such incorporation in all species of animals.

The claims read on any and all transgenic embryos including humans. Furthermore, it is unpredictable if transgenic embryos of any and all species can be created using the method of the claimed invention. The art of transgenics is not a predictable art with respect to transgene behavior. Without evidence to the contrary, transgene expression of different species of transgenic animals varies according to the particular host species. It is not possible to predict successful transgenesis in any and

all embryos using the method of the claimed invention since the specification has only provided one working model, the mouse, which demonstrates successful transgenesis. This observation is supported by Mullins et al. (Journal of Clinical Investigations, 1996) who report on transgenesis in the rat and larger mammals. Mullins et al. state that "a given construct may react very differently from one species to another." See page S39, Summary. Wall et al. report that "transgene expression and the physiological consequences of transgene products in livestock are not always predicted in transgenic mouse studies." See page 62, first paragraph. Kappel et al. (Current Opinion in Biotechnology, 1992) disclose the existence of inherent cellular mechanisms that may alter the pattern of gene expression such as DNA imprinting, resulting from differential CpG methylation (page 549, column 2, 3rd full paragraph). Strojek and Wagner (Genetic Engineering, 1988) pointed out that a high degree of expression of a transgene in a mouse is often not predictive of high expression in other species, including pigs and rabbits, because, for example, the cis acting elements may interact with different trans-acting factors in these other species (paragraph bridging pages 238-239). The correlation of successful in vitro fertilization of various animal species, as taught in the prior art, to the method of the claimed invention is not justified since there may be additional inventive steps, not disclosed in the specification, which are species specific but necessary for successful transgenesis. Given such species differences in the expression of a transgene, one of skill in the art would have been required to undergo undue experimentation to determine which promoter, enhancer, intron, exon, and

transgene construct would produce the desired phenotype in any and all animals. Thus in view of the breadth of the claims, the lack of guidance provided by the specification, the absence of working examples, and the unpredictability of the art, one of skill in the art would have required an undue amount of experimentation to create any transgenic embryo by any and all methods of transferring a sperm head and exogenous DNA into oocytes except by microinjection of a DNA-sperm head complex using a piezo-electrically actuated apparatus as a method of transfer into unfertilized mouse oocytes, without a reasonable expectation of success.

Claim 16 reads on any and all mammals including humans. Furthermore, the specification is not enabling for the creation of any and all transgenic mammals using the method of the claimed invention for the reasons provided above.

Claim 17 reads various groups of mammals including humans as written. Humans are primates. The specification is not enabling for the creation of transgenic mammals, from the species of claim 17, using the method of the claimed invention for the reasons provided above.

Claims 18-20 are drawn to various animal species as written. The state of the prior art is such that no microinjection of bird oocytes has been successful to date due to the physical consistency of the avian oocyte. Furthermore, birds ovulate only once every twenty-four hours making it difficult to obtain oocytes. The specification is not enabling for the creation of transgenic non-human animals of claims 18-20 using the method of the claimed invention for the reasons provided above.

Therefore, in view of the quantity of experimentation necessary to determine the parameters necessary for the creation of transgenic embryos of any and all species using the method of the claimed invention; the lack of direction or guidance provided by the specification with respect to all methods of "co-inserting" a sperm head and exogenous DNA into oocytes, and creation of transgenic embryos of any and all species using the method of the claimed invention; the absence of working examples for the demonstration or correlation with respect to the production of transgenic animals of any and all species using the method of the claimed invention, and all methods of "co-inserting" a sperm head and exogenous DNA into oocytes; the unpredictable state of the art with respect to the creation of any and all transgenic animals using the method of the claimed invention; the breadth of the claims drawn to any and all animals and all methods of "co-inserting" a sperm head and exogenous DNA into oocytes, it would have required undue experimentation for one skilled in the art to use the method of the claimed invention.

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, and 4-21 rejected under 35 U.S.C. 103(a) as being unpatentable over Lavitrano et al (1989, Cell, 57:717-723) in view of Kuretake et al (1996, Biol of Reprod., 55: 789-795).

Lavitrano et al disclose a method of creating a transgenic mouse comprising incubating live, intact mouse sperm with plasmid DNA (page 718, lines 1-3), fertilizing mouse oocytes with the sperm-DNA complex, transferring the resulting embryos to foster mothers (page 718 column 2 paragraph 1 lines 1-8), and analyzing the offspring by Southern blot of tail DNA for the presence of the transgene (paragraph 2 lines 1-3). Germline transmission of the transgene was established (page 720 column paragraph 1 lines 1-4 and figure 7 page 721). Lavitrano et al disclose that mouse spermatozoa can capture foreign DNA and suggest that DNA can be transferred into egg cell at fertilization (page 721 Discussion lines 1-5).

Lavitrano et al do not expressly disclose the following method step for creating a transgenic mouse: microinjecting a complex of DNA and membrane disrupted or demembranated sperm heads into unfertilized mouse oocytes.

However, at the time the claimed invention was made, Kuretake et al disclose a method of in vitro fertilization of mouse oocytes comprising microinjecting membrane disruped or demambranated sperm heads into mouse oocytes (page 790 column 2 paragraphs 2-3). Kuretake et al disclose that live sperm can be either sonicated with triton x-100 to separate the head (page 789 bridging 799 paragraphs 1-2) from the tail. Kuretake further disclose that "damage to the sperm plasma membrane increases the

fertilization rate by ICSI [intracytoplasmic sperm injection]" (page 789 paragraph 2 lines 5-6).

At the time the claimed invention was made, it would have been *prima facie* obvious to one of ordinary skill in the art to combine the teachings of Lavitrano et al and Kuretake et al and design a method of creating a transgenic mouse comprising microinjecting demembranated or membrane disrupted sperm heads complexed with DNA into mouse oocytes, transferring the embryos to a foster mother, analyzing the offspring for the presence of the transgene, and breeding the transgene positive mice with wild-type mice to test for germline transmission of the transgene to the F1 progeny. One would have been motivated to do this because Kuretake et al disclose that sperm with a damaged plasma membrane increase the fertilization rate by ICSI (page 789 paragraph 2 lines 5-6). An increase in fertilization rate could also increase the percentage of transgenic mice obtained since the method requires that the exogenous DNA is transferred into the egg cells by the membrane disrupted or demembranated sperm heads at fertilization.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner(s) should be directed to Peter Paras, Jr., whose telephone number is 703-308-8340. The examiner can normally be reached Monday-Friday from 8:30 to 4:30 (Eastern time).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jasemine Chambers, can be reached at 703-308-2035. The FAX phone number for art unit 1632 is 703-308-0294.

Inquiries of a general nature or relating to the status of the application should be directed to the group receptionist whose telephone number is 703-308-0196.

Peter Paras, Jr.
Art Unit 1632

Karen M. Hauda
Karen M. Hauda
Patent Examiner